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CURRENT OPINION

Recent developments in understanding protein needs – How much and what kind should we eat?[1]

Paul B. Pencharz, Rajavel Elango, and Robert R. Wolfe

Abstract: A novel method has been developed to determine protein requirements, which is called indicator amino acid oxidation (IAAO). This technique has been validated by comparison with the "gold standard" nitrogen balance. Using IAAO we have shown that minimum protein requirements have been underestimated by 30%–50%. The National Academy of Sciences has for macro-nutrients proposed "Acceptable Macronutrient Distribution Ranges", which for protein is 10% to 35% of total energy. In practice, we suggest 1.5–2.2 g/(kg·day) of a variety of high-quality proteins.

Key words: protein, protein recommendations, protein requirements, amino acid requirements, protein quality, optimal protein intakes, energy, growth, lean body mass.

Résumé : Une nouvelle méthode est élaborée pour la détermination des besoins protéiques; il s'agit de l'indicateur de l'oxydation des acides aminés (« IAAO »). Cette méthode est validée par comparaison au bilan azoté, la norme de référence. En utilisant l'IAAO, on démontre que les besoins protéiques sont sous-estimés de 30–50 %. La « *National Academy of Sciences* » suggère pour les macronutriments « l'Étendue des valeurs acceptables pour les macronutriments » dans laquelle les protéines représentent de 10 à 35 % de l'énergie totale. Dans la pratique, nous suggérons 1,5–2,2 g/(kg·jour) de protéines de haute qualité et de sources variées. [Traduit par la Rédaction]

Mots-clés : protéines, recommandations pour les protéines, besoins protéiques, besoins en acides aminés, qualité des protéines, apports protéiques optimaux, énergie, croissance, masse maigre.

Introduction

This brief synopsis highlights recent developments in understanding protein needs based on presentations at the 2015 Canadian Nutrition Society conference, *Advances in Protein Nutrition across the Lifespan*. Understanding of protein needs is based upon first defining the minimum amount required for health, which is described in the current Dietary Reference Intakes (DRI), by the terms estimated average requirement (EAR) and recommended dietary allowance (RDA) (Institute of Medicine 2005). The RDA is intended to cover minimum protein needs for 97.5% of the healthy population. Second, protein needs are based on defining the safe upper limits. The Institute of Medicine (2005) report goes on, based on the first 2 steps, to define "Acceptable Macronutrient Distribution Ranges" (AMDR). The current AMDR for protein is 10% to 35% of total energy for adults. The present review outlines the work published since the Institute of Medicine (2005) recommendations and is focused on a novel method to determine the minimum amount of protein required for health. The work presented covers only healthy human beings and ranges from school aged children to the elderly. The question of "how much and what type of protein should we eat?" is also explored.

A review of current evidence on protein requirements to prevent age-related sarcopenia and to promote healthy weight management and peak athletic performance, also based on presentations at the 2015 Canadian Nutrition Conference on protein, has been published by Phillips et al. (2016).

Until quite recently protein and amino acid requirements were determined using nitrogen balance (namely food nitrogen intake minus nitrogen excreted). The drawbacks with nitrogen balance are that a minimum of 3 days is needed per level of test intake and 7–10 days of adaptation are needed to each intake of protein or amino acid. In addition, complete collection and quantification of all sources of nitrogen excretion (mostly in urine and faeces) is difficult. More fundamentally, the nature of the calculation of nitrogen balance is likely to result in wide variability because nitrogen intake and nitrogen excretion are much larger numbers than the difference between them (i.e., nitrogen balance). Hence alternative methods have been developed based on carbon oxidation.

Currently, a suitable alternative is the indicator amino acid oxidation (IAAO) method (Elango et al. 2008). Briefly, an essential amino acid (usually phenylalanine, lysine, or leucine) is labelled with the stable isotope carbon-13 (^{13}C) and the appearance of the label in breath carbon dioxide ($^{13}CO_2$) is used as an indicator of

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protein or amino acid requirement. Graded intakes of protein or amino acids are fed to the experimental subjects and a breakpoint is defined as a reflection of requirement. Adaptation of only 3 to 4 h is needed since IAAO is dependent upon the rapidly turning over pools of tRNA-test amino acid from which proteins are synthesized (Elango et al. 2012*a*). Evidence from the application of the IAAO technique in various groups suggests current recommendations substantially underestimate minimum protein requirements throughout the lifecycle.

Protein and amino acid requirements

In 1900 a German Scientist, Voit, reported the protein requirement as 1 g/(kg·day) based on what highly productive factory workers ate. The World Health Organization Committees estimated mean protein requirements as 0.66 g/(kg·day) with a population requirement of 0.8 g/(kg·day), based on nitrogen balance data analyzed using mono-linear regression. A reanalysis of the published nitrogen balance data using 2-phased linear crossover analysis resulted in a mean requirement of 0.91 g/(kg·day) and a population estimate of 1.0 g/(kg·day), which are identical to that of Voit 100 years earlier (Humayun et al. 2007). This estimate was confirmed using the carbon oxidation based method, IAAO, with a mean estimate of 0.93 g/(kg·day), which is essentially the same as the reanalysis of the nitrogen balance data (Humayun et al. 2007). Concern has been raised that this experimental design used a complete and balanced mixture of amino acids, instead of intact protein; and hourly meals, instead of standard meals every 4 h. An Asian group (Tian et al. 2011) has conducted studies in young Chinese women using intact protein and meal feeding and applied the IAAO method to determine protein requirement. The results obtained for mean and population safe protein requirement of 0.91 and 1.09 g/(kg·day) are comparable to the estimates obtained by Humayun et al. (2007). Once it is appreciated that the dietary function of protein is to provide the 20 amino acids for which tRNA exists (Institute of Medicine 2005), the observation of Tian et al (2011) are not surprising. Studies in the elderly (Rafii et al. 2015; Tang et al. 2014) using the carbon oxidation approach obtained essentially identical estimates on a body weight basis.

During pregnancy, protein needs are different depending on the stage of gestation, with early stages requiring 1.2 g/(kg·day) and later stages of pregnancy requiring 1.5 g/(kg·day) (Stephens et al. 2015). Moreover, a mean protein requirement of 1.3 g/(kg·day) has been reported in preadolescent school-aged children (Elango et al. 2011). The protein intakes of economically challenged children are much less, around 0.9 g/(kg·day) and growth stunting is a major issue in the developing world. Feeding growing farm animals insufficient protein also results in growth restriction. Hence, there is strong evidence that protein needs in children have been underestimated by around 50% and results in marked growth retardation in the developing world (Elango et al. 2010, 2011). These observations have important implications for public health and world agriculture.

Children living in the developing world also have a diet that is limiting in the essential amino acid lysine. It had been hypothesized that these populations may be able to adapt and thrive on a lower lysine intake than that needed in the developed world. To test this question, the lysine needs of school-aged children in Toronto, Canada, and in Bangalore, India, were measured and shown to be identical (Pillai et al. 2010). Moreover, the lysine requirements in moderately malnourished Indian children increased by 20% because of gut parasite infection (Pillai et al. 2015). Thus, healthy children worldwide have similar lysine requirements, while the presence of gut parasites increases the need for lysine.

The noninvasive nature of the IAAO technique has also allowed for the study of amino acid needs in other vulnerable individuals. Thus, direct measurement of the limiting amino acids requirements in inborn errors of amino acid metabolism, such as phenylketonuria and maple syrup urine disease, have been obtained for the first time (Pencharz and Ball 2006). IAAO has also been used to determine requirements for branched chain amino acids, which are increased in children with liver disease (Mager et al. 2006). This is useful since it makes the prevention of malnutrition and growth failure possible in children with chronic liver disease. Amino acid needs in premature infants, especially those needing parenteral nutrition, have yet to be completely defined. Amino acid requirements during pregnancy and lactation also need to be defined.

How much protein should we eat?

Current dietary guidelines include a number of different recommendations for dietary protein (as outlined in supplementary Table S1[2]). Lack of clarity and apparent contradictions in the current guidelines have resulted in uncertainty in practice. Some of the confusion may be alleviated by understanding the basis for the various recommendations and by relabelling dietary guidelines in terms of the recommended minimal and flexible intake.

North American adult habitual protein intakes average 16% of energy (1.68 g/(kg·day)) and range up to 23% of energy (2.4 g/(kg·day)). These values are derived from the United States Department of Agriculture (USDA) and Health Canada data contained as supplementary table to the Institute of Medicine, Macronutrient DRI (Institute of Medicine 2005). The concept of an ADMR also comes from the Macronutrient DRI.

Current evidence indicates that most adults will benefit from intakes above the RDA of 0.8 g protein/(kg·day). The nitrogen balance approach used to determine the EAR of 0.66 g protein/(kg·day), (and thus the RDA) defines a minimal level of protein intake needed to avoid a deficiency (Institute of Medicine 2005). This approach does not consider protein intake in relation to physiological functions responsive to the level of dietary protein intake or the relation of protein to the intake of other macronutrients. Thus, although the RDA is often interpreted as a target for the desired level of intake, in reality it better reflects a minimal amount that will prevent symptoms of deficiency in most individuals.

It is important to remember that we eat protein in the context of complete meals. The DRI report also presents recommendations for macronutrient intakes as the AMDR. For the sake of easy comparison with the other values, the AMDR for protein of 10%–35% of total caloric intake for adults can be converted into g protein/(kg·day) by assuming a caloric expenditure of 42 kcal/(kg·day) and body weights of 57 kg for women and 70 kg for men (Institute of Medicine 2005). Based on this, the AMDR recommends a protein intake of 1.05 to 3.67 g/(kg·day) for adults (i.e., well above the RDA). Analysis of the USDA "My Pyramid" reveals a recommended protein intake of 17%–21% of caloric intake (Fulgoni 2008), or 1.78–2.20 g/(kg·day) for the energy expenditure and body weight assumed in the DRIs.

Beyond zero nitrogen balance

When nitrogen intake exceeds the amount required to achieve zero nitrogen balance, a progressively positive nitrogen balance (i.e., nitrogen intake greater than nitrogen output) results. It has been assumed that positive nitrogen balance values in adults are artifacts and therefore not considered in the estimation of the EAR (Millward 2012), but the justification for ignoring positive values is largely that the results do not fit the preconceived model. However, when the nitrogen balance data, collected from studies

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/apnm-2015-0549.

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conducted around the world, were reanalyzed (including that with positive balance) using 2-phase linear regression crossover analysis to determine EAR and RDA, the mean calculated values were 0.93 and 1.2 g protein/(kg·day), respectively (Elango et al. 2010). These values were approximately 50% higher than the corresponding values published in the DRI report.

Experimental results from a variety of sources indicate that net protein balance (i.e., protein synthesis minus protein breakdown) increases linearly as a function of amino acid availability (Deutz and Wolfe 2013). The principal response to an increase in dietary protein at levels below the EAR is an increase in net protein balance because of stimulation of protein synthesis. Rates of protein intake greater than the EAR not only stimulate protein synthesis, but progressively inhibit protein breakdown.

A number of outcome studies also suggest that dietary protein intake greater than the RDA may increase lean body mass and improve physical function. For example, supplementation of the normal diet twice per day with a mixture of essential amino acids equivalent to the amount in approximately 30 g of high-quality protein, without any alteration in normal dietary intake or exercise, increased lean body mass, strength, and functional test scores in free-living, healthy, elderly subjects (Børsheim et al. 2008; Dillon et al. 2009). Moreover, acute stimulation of muscle protein fractional synthetic rate by amino acids translated to a significant amelioration of the decline in functional status in healthy elderly that normally occurred after 10 days of enforced bed rest (Ferrando et al. 2010). Supplementation of the diet with the same mixture of amino acids, along with a small amount of carbohydrate, also decreased the extent of loss of lean body mass and muscle strength in response to enforced bed rest in healthy young subjects (Paddon-Jones et al. 2004). Increased protein intake has also been demonstrated to have beneficial effects on muscle mass in other studies in older adults (Chevalier et al. 2003).

Estimating an optimal range of protein intakes for adults

When estimating an optimal amount of dietary protein it is important to consider the composition of the entire diet. In an adult with a normal rate of energy expenditure (42 kcal/(kg·day)) (Institute of Medicine 2005), the RDA for dietary protein meets less than 10% of caloric requirement. The balance of the diet must be composed of carbohydrate and fat (or alcohol). The beneficial effects of dietary protein and the potential adverse effects of excessive intakes of carbohydrate or fat also suggest that the optimal level of protein intake exceeds the RDA for protein. The question is: exactly how much protein should we eat?

The caloric values of the RDAs for protein and carbohydrate plus the recommended minimal fat intake sum to only about 40% of total caloric expenditure (Institute of Medicine 2005). Thus, the complete diet can be thought of as consisting of 2 components: a minimal intake for protein, carbohydrates, and fat and the flexible intake that comprises the difference between the caloric values of the RDAs or minimal intake and total caloric intake. If just 10% of the flexible intake is protein, this would correspond to 0.7 g/(kg·day), added to the RDA of 0.8 g/(kg·day), for a total of 1.5 g/(kg·day). If 20% of the flexible intake comprises protein, this would correspond to 1.4 g/(kg·day), or a total protein intake of 2.2 g/(kg·day). These values represent conservative estimates of the optimal contribution of protein to the flexible intake of dietary calories, and fall well within the range of dietary protein intakes recommended by the AMDRs and the USDA Dietary Guidelines. There are no known adverse effects of this level of protein intake in normal individuals (Institute of Medicine 2005; Phillips et al. 2016). Therefore, we propose that in practice, 1.5–2.2 g/(kg·day) constitutes a reasonable recommendation for the amount of protein that should be eaten by adults as part of a complete diet.

What type of protein should we eat?

The assumption underlying recommendations for dietary protein is that it consists of "high-quality" protein. The term protein quality refers to the balance of the amino acids, the digestibility of the protein to release the amino acids for absorption, and the availability of the absorbed amino acids for protein synthesis. The case of lysine provides an example of the importance of considering the amount of amino acids available for metabolic processes, most notably protein synthesis. Cooking can denature lysine and hence make the absorbed lysine not available for protein synthesis (Food and Agriculture Organization 2013). The Digestible Indispensable Amino Acid Score (DIAAS) recently proposed by the Food and Agriculture Organization (FAO 2013) provides a more accurate estimate of protein quality than the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which was previously used to quantify protein quality. PDCAAS is based on the ratio of the first-limiting essential amino acid in the test protein to the reference protein. The values were truncated at 1, although high-quality proteins have PDCAAS well above 1. The principal advantages of DIAAS are that (*i*) the true ileal digestibility of individual amino acids in the test protein is taken into account, and (*ii*) the values are not truncated. Values for DIAAS are expressed as the percent of the dietary requirement for each essential amino acid met by ingestion of 0.66 g of the test protein/(kg·day), and the lowest DIAAS is considered the DIAAS of the test protein. DIAAS scores for animal proteins such as milk, eggs, and beef are well above 100%, whereas vegetable proteins generally fall below 80% with the exception of soy.

As stated by the FAO (2013), our current methods and understanding of protein quality in humans is very limited. Conversely, in agriculture, the protein quality of animal feeds is better understood. Hence, we are currently conducting studies in pigs and humans to develop more practical and valid methods to determine the quality of plant protein in humans (Elango et al. 2012*b*). Cereals protein are limited in lysine (all), threonine (most), and tryptophan (maize), and legumes are sufficient in lysine, threonine, and tryptophan but are limited in sulphur amino acids. Hence complementation (mixtures) of cereals and legumes are being evaluated to determine how to achieve the best possible plant protein mixtures.

The discrepancies in quality between animal and plant proteins become dramatic when the energy equivalents of the food sources are accounted for in the expression of quality. Caloric requirement to meet essential amino acid requirements for plant proteins are considerably greater than required by the ingestion of animal proteins. This is an important consideration given that obesity and sarcopenia (i.e., loss of lean body mass with age) are two of today's largest public health challenges. As outlined by Phillips et al. (2016), protein intakes above the current RDA (within the AMDR) can help to improve satiety and support weight management efforts and may help to prevent age-related sarcopenia as people age and have no known adverse impacts on health. There is an urgent need to reevaluate protein recommendations to ensure they reflect recent developments in understanding protein requirements.

Take-home points

- Recent evidence indicates the current RDAs substantially underestimate minimum protein requirements throughout the lifespan.
- The AMDR of 10% to 35% of calories from protein for adults allows considerable flexibility to recommend protein intakes above the current RDA.
- In practice, 1.5 to 2.2 g/(kg·day) of high-quality protein constitutes a reasonable recommendation for adults as part of a complete diet.
- High-quality animal proteins require far less energy intake to meet essential amino acid needs than lower quality plant proteins.

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Conflict of interest statement

Paul Pencharz and Rajavel Elango declare that there are no conflicts of interest. Robert Wolfe has received research grants from Abbott, Baxter, and the National Cattlemen's Beef Association. He has received honoraria for speaking from the National Cattlemen's Beef Association.

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